



Correlation of *KIF3A* and *OVOL1*, but not *ACTL9*, with atopic dermatitis in Chinese pediatric patients



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ABSTRACT

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease in Chinese pediatric patients. To date, the genetic susceptibility to AD in this population has not been fully clarified. Three single nucleotide polymorphisms have previously been associated with AD in Europeans, rs2897442 (*KIF3A*), rs479844 (*OVOL1*) and rs2164983 (*ACTL9*). To verify the correlation between AD and these three SNPs in the Chinese pediatric population, we conducted a case–control study including 235 pediatric patients with AD and 200 health controls. We confirmed the correlation between rs2897442 and rs479844 and AD in this population at both the genotype and allele levels. Statistical analysis showed that the C allele of rs2897442 is associated with an increased risk of developing AD, while the A allele of rs479844 is associated with a reduced risk. No correlation between rs2164983 and AD was identified. Our study indicates that *KIF3A* and *OVOL1* are involved in the development of AD in the Chinese pediatric population.

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1. Introduction

Atopic dermatitis (AD), or eczema, is one of the most common chronic inflammatory skin diseases and is characterized by dry skin, pruritus and erythematous lesions (Boguniewicz et al., 2006; Bieber, 2008; Flohr et al., 2005). There has been a dramatic increase in the morbidity of AD with prevalence rates reaching 20% in children and 3% in adults over the last decade (Bieber, 2008; Wolter and Price, 2014; Hanifin and Reed, 2007). Although the pathogenesis of AD remains largely undefined, a complex interaction between genetic and environmental factors is thought to contribute to the disease onset (Leung et al., 2004; Hudson, 2006; Wuthrich et al., 2007). *FLG*, which was the first susceptibility gene to be identified in AD (Palmer et al., 2006; O'Regan et al., 2009) encodes the FLG protein that plays an essential role in maintenance of the skin-barrier function.

Genetic mutations in *FLG* were found to be significantly associated with the risk of AD and elevated immunoglobulin E levels (Nemoto-Hasebe et al., 2009; Enomoto et al., 2008). Subsequently, a number of genes, for instance of *TMEM232/SLC25A46* and *TNFRSF6B/*

ZGPAT (Kim et al., 2014), have been implicated in the dysfunction of the skin-barrier and immune regulation in AD patients. Identification of these genes would further elucidate the pathogenesis of AD and provide an important basis for improved therapeutics and disease management (Kim et al., 2014; Zhang et al., 2014; Sasaki et al., 2013; Hon et al., 2013).

In a recent genome-wide association meta-analysis, Paternoster et al. (2012) identified rs2897442 of *KIF3A*, rs479844 of *OVOL1* and rs2164983 of *ACTL9* as new susceptibility loci with genome-wide significance. A case–control study based on an Italian population performed by Lepre et al. (2013) showed a significant difference in the frequency of rs2897442 between cases and controls, but no statistically significant association between AD and the other two SNPs.

In this study, therefore, we aimed to analyze the association between genetic variations at the *OVOL1*, *ACTL9* and *KIF3A* gene loci and AD susceptibility in Chinese pediatric AD patients and healthy controls.

2. Materials and methods

2.1. Cases and controls

This case–control study included 235 pediatric patients with AD and 200 healthy controls. The patient population consisted of 126 males and 109 females (average age, 12 y; range, 6–15 y). All patients were diagnosed as AD by at least two independent dermatologists in the Department of Dermatology in Huashan Hospital, Fudan University (China).

Abbreviations: AD, Atopic dermatitis; SNP, Single nucleotide polymorphism; PCR, Polymerase chain reaction; HRM, High resolution melting.

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Detailed clinical records were available for all patients, including details of age, sex, time of disease onset; patients were then classified into subgroups according to these clinical records. The inclusion criterion was a diagnosis of AD according to standard criteria.

The control group comprised 200 healthy individuals (115 males and 85 females; average age, 10 y; range, 7–13 y). The absence of AD or other inflammatory conditions and autoimmune diseases in this group was confirmed by screening in physical examinations at our hospital. Each individual presented with a complete blood count, blood glucose level, renal and liver function, erythrocyte sedimentation rate and C-reactive protein within the normal range.

This study was approved by the Ethics Committee of Huashan Hospital, Fudan University and all participants gave written informed consent to participation in genetic studies.

2.2. Extraction of genomic DNA

Genomic DNA was isolated from freshly obtained peripheral blood using the QIAamp DNA Blood Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions.

2.3. High resolution melting analyses

PCR amplification was performed using GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA), and the high resolution melting (HRM) analysis was performed using the Rotor-Gene Q™ (Qiagen, Mortlake, Australia). Primer sequences were designed to amplify a small fragment spanning the SNPs rs2897442, rs479844 and rs2164983 single nucleotide polymorphisms (SNPs) and to avoid the presence of other sequence variations. PCR amplification was performed in a 20 µl reaction volume containing 50 ng DNA, 10 µl Premix Taq Hot Start polymerase (TaKaRa, Dalian, China), 0.25 mM primers and 5 mM SYTO-9 dye (Invitrogen, Carlsbad, CA, USA). HRM analysis was performed immediately after PCR amplification by ramping from 75 °C to 90 °C with a temperature gradient of 0.1 °C per second. The normalized melting curves of the PCR products were used for genotyping. HRM data were analyzed using the Rotor-Gene Q software.

2.4. Direct sequencing

Selected samples identified in the HRM analysis were subjected to direct sequencing. Amplicons were gel-purified using the QIAquick gel purification kit (Qiagen, Valencia, CA, USA). DNA sequencing analysis was performed using an ABI PRISM 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

2.5. Statistical analyses

Genotype and allele frequency distributions were compared using Chi-squared tests. In addition, the odds ratio (OR) and 95% confidence interval (CI) values were calculated with the use of logistic regression. *P*-values of less than 0.05 were considered to indicate statistical significance. All data were evaluated using Stata 10.0. Statistical power was calculated using <http://www.stat.ubc.ca/~rollin/stats/ssize/b2.html>.

3. Results

3.1. Genotyping with HRM analysis and direct sequencing

A total of 235 AD cases and 200 healthy control individuals were screened for allele distribution of the rs2897442, rs479844 and rs2164983 SNPs. HRM analysis and direct sequencing were successfully established for genotyping these three SNPs.

3.2. Associations between rs2897442, rs479844, rs2164983 SNPs and susceptibility to AD

A significant difference in the allele distribution of *KIF3A* rs2897442 was observed between AD patients and healthy controls (Table 1; *P* = 0.006). There was also a significant difference in the genotype frequency of rs479844 in *OVOL1* between AD patients and healthy controls (Table 1; *P* = 0.048), but not in the genotype of frequency of *ACTL9* rs2164983 between these groups (Table 1; *P* = 0.712). However, the low statistical power (<0.8) for these three SNPs due to the relatively small sample size should be noted.

Logistic regression analyses showed that the C allele of rs2897442 was associated with an increased risk of developing AD (OR = 1.74, 95% CI: 1.14–2.66), while the A allele of rs479844 was associated with a reduced risk of developing AD (OR = 0.68, 95% CI: 0.48–0.96).

4. Discussion

Atopic dermatitis (AD) is one of the most common chronic inflammatory skin diseases in the pediatric population (Flohr and Mann, 2014) and accumulating evidence indicates a strong genetic basis in this disorder (Morar et al., 2006; Barnes, 2010; Busmann et al., 2011).

In this case–control study, we investigated the relationship between the rs2897442 (*KIF3A*), rs479844 (*OVOL1*) and rs2164983 (*ACTL9*) SNPs and AD in 235 pediatric AD patients and 200 healthy controls. Our findings provide preliminary evidence of the involvement of rs2897442 and rs479844, but not rs2164983, in the pathogenesis of AD in the Chinese pediatric population. The effect alleles of rs2897442 (C) and rs479844 (A) demonstrated a risk OR of 1.74 [95% CI: 1.14–2.66] and a protective OR of 0.68 [95% CI: 0.48–0.96]. It is important to note that the relatively small number of cases and controls included in this study represents a limitation that decreases the statistical power of our data.

Rs2897442 is located in intron 8 of *KIF3A*, encoding a motor subunit of kinesin-2, which is an important component in primary cilia formation. This gene is also implicated in Hedgehog signaling and β -catenin-dependent Wnt signaling in the regulation of the expression of various genes that influence cell proliferation and apoptosis (Mosimann et al., 2009; Goetz and Anderson, 2010). Notably, *KIF3A* is located in the 5q31 region, which is characterized by a complex linkage disequilibrium (LD) pattern and contains cytokine clusters and immune-related genes thought to play an important role in the development of several autoimmune or inflammatory diseases, including asthma, psoriasis, and Crohn's disease (Chang et al., 2008; Kim et al., 2011; Corbit et al., 2008). A complex pattern of LD is also associated with rs479844 (at 11q13.1), which is located upstream (<3 kb) of *OVOL1*, a gene belonging to a highly conserved family of genes involved in regulation of the development and differentiation of epithelial tissues (Li et al., 2005; Dai et al., 1998; Teng et al., 2007). *OVOL1*, which acts as a c-myc repressor in keratinocytes, could be activated by the β -catenin–LEF1 complex during epidermal differentiation, and represents a downstream target of the Wnt and TGF- β /BMP7–Smad4 developmental signaling pathways (Nair et al., 2007). Apart from their role in the organogenesis of skin and skin appendages, these pathways are also involved in the postnatal regulation of epidermal proliferation and differentiation.

In this study, statistically significant differences in the distribution of rs2897442 in *KIF3A* and rs479844 in *OVOL1* were identified between AD patients and healthy control individuals at both the genotype and allele levels, while the difference in the distribution of rs2164983 variants of *ACTL9* did not reach the level of statistical significance. The detailed function of *ACTL9* remains largely unknown although it is thought to function within the cytoskeleton to maintain epithelial morphology and cell migration capacity. Our findings and those of others (Paternoster et al., 2012; Lepre et al., 2013) indicate that rs2164983 plays a relatively minor role in the pathogenesis of AD.

Table 1

Genotype and allele distribution of the rs2897442, rs479844, rs2164983 in AD patients and health control.

SNP ID	Genotype (frequencies)			P-value	Allele (frequencies)		P-value	OR (95% CI)
rs2897442	CC	CT	TT	0.006	C	T	0.0103	C: 1.74 (1.14–2.66)
Cases	194 (0.83)	41 (0.17)	0		429 (0.91)	41 (0.09)		
Health controls	143 (0.72)	57 (0.28)	0		343 (0.86)	57 (0.14)		
rs479844	AA	AG	GG	0.048	A	G	0.0282	A: 0.68 (0.48–0.96)
Cases	134 (0.57)	98 (0.42)	3 (0.01)		366 (0.78)	104 (0.22)		
Health controls	137 (0.69)	61 (0.30)	2 (0.01)		335 (0.84)	65 (0.16)		
rs2164983	CC	CA	AA	0.922	C	A	0.9297	C: 0.98 (0.68–1.42)
Cases	162 (0.69)	73 (0.31)	0		397 (0.84)	73 (0.16)		
Health controls	137 (0.69)	63 (0.31)	0		337 (0.84)	63 (0.16)		

CI, confidence interval; OR, odds ratio.

Our findings are in agreement basically with a previous report of a meta-analysis study (Paternoster et al., 2012) conducted in a large European population including 11,025 cases and 40,498 controls, which showed a significant association of the rs2897442, rs479844 and rs2164983 polymorphisms with AD. Furthermore, as shown in Table 1, the C allele of rs2897442 was identified with a frequency of 0.91 in cases and 0.86 in controls, reaching a risk OR of 1.74 [95% CI: 1.14–2.66]. In the European study, the C allele of rs2897442 was also identified as a risk allele (OR of 1.11 [95% CI: 1.07–1.15]). The A allele of rs479844 was identified with a frequency of 0.78 in cases and 0.84 in controls, achieving a protective OR of 0.68 [95% CI: 0.48–0.96]. Consistently, the A allele of rs479844 demonstrated a protective OR of 0.88 [95% CI: 0.85–0.91] in the European population. The A allele of rs2164983 showed an ambiguous OR of 0.98 [95% CI: 0.68–1.42] in our population compared to a risk OR of 1.16 [95% CI: 1.10–1.22] in the previous European study.

Generally speaking, all CIs of these three SNPs in our study are almost overlapping with that original meta-analysis in the European population, even for rs2164983. Lack of statistical power is likely to be a major issue in the replication of all the SNPs. The low minor allele frequency of rs2164983 and the relatively small size of our population kept us from achieving significance for this one particular SNP. Thus, larger studies are required to confirm the effects of such polymorphisms on the clinical manifestations of AD in Chinese children. On the other hand, these observations also provide compelling evidence of the complex pathogenesis of AD, with numerous genetic factors implicated in the development of this disease. For these three SNPs, according to the statistically significant differences in the distribution and the OR values, rs2897442 might exert larger effect on the pathogenesis of AD in the Chinese pediatric population than the other two SNPs.

In conclusion, the findings of this study indicate that rs2897442 of *KIF3A* and rs479844 of *OVOL1* are involved in the pathogenesis of AD in the Chinese pediatric population.

Conflict of interest

The authors declare that they have no conflicts of interest, financial or otherwise, related to the publication of this study.

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